The relation of morphology and affinity maturation in germinal centers

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Abstract: The specific morphology of germinal centers is analyzed in the context of the optimization of the humoral immune response. The relevance of dark and light zones for the affinity maturation process is investigated in the framework of a theoretical model for the germinal center reaction. Especially, it is shown that an intermediate appearance of dark zones in germinal center reactions is advantageous for the process of antibody optimization.

Keywords: Immune system, germinal center, morphology, affinity maturation, lymphocytes, centroblasts, centrocytes, B-cell kinetics.

1 Introduction

An important part of the humoral immune response is the germinal center (GC) reaction. GCs are responsible for an optimization process of antibodies with respect to a specific antigen. This process is called affinity maturation: During the GC reaction new plasma cells are generated which secern antibodies of considerably higher affinity to the antigen.

The GC reaction is initiated by antigen-activated B-cells that migrate into the follicle system. Here, they start to proliferate in the environment of follicular dendritic cells (FDCs). The initiation is believed to be of oligoclonal character, i.e. the number of seeder B-cells is small and of the order of three[1,2,3,4]. After three days of fast monoclonal expansion – the total number of proliferating B-cells (centroblasts) reaches about 12000 – a phase of somatic hypermutation is started.[5,6,7,8] The diversity of encoded antibodies is enhanced in this way. The centroblasts differentiate into antibody-presenting centrocytes[9] and an apoptotic process is initiated. However, they have the possibility to get into interaction with the antigen-presenting FDCs and with T-helper cells. It is believed that this interaction depends on the affinity of antibody and antigen, and that those centrocytes which successfully bind the antigen are rescued from apoptosis.[10,11,12,13,14,15] This provides a more-step selection process[16] of those B-cells with high affinity to the antigen. Positively selected B-cells further differentiate into plasma- and memory-cells (shortly denoted as output cells). In this way the answer of the immune system is optimized with respect to the antigen.

The GC shows a very specific morphology. The proliferating and mutating centroblasts are collected in the dark zone. Centrocytes and FDCs build the light zone. Such zones have been observed in experiments.[2,17] Nevertheless, it is still unclear, how long the dark zone remains present during a GC reaction. The total duration of a GC reaction is about 21 days.[2,3,18] Dark zones have been observed to appear at day 4 and to vanish at day 8.[17] However, there also exists evidence for dark zones of longer duration.[2]

In the present article a possible correlation of the GC morphology and affinity maturation is investigated. To this end, a mathematical model[19] is used which includes the cell interactions as well as their spatial distribution and movement. To our knowledge this model is up to now the only attempt to simulate the morphological organisation and dynamics of the GC. Other mathematical models of the GC did not include a spatial resolution of the GC.[20,21,22,23,24,25]

The principles of the model are summarized and shortly described in Sec. 2. All principles are in narrow connection to the present experimental knowledge, and the model language is formulated in terms of physiological quantities. The latter are determined by corresponding measurements. The resulting values are used in this article. An analysis of the robustness of the results has been performed

previously.[19,20] Here, we aim to report one major outcome of the model including an optimized statistic of the analysis (Sec. 3). In addition we propose an interpretation and possible implications of the results (Sec. 4).

2 Methods

A short description of a previously introduced mathematical model for the morphological organisation and cell dynamics of the GC[19] is provided in this section. The GC is simulated on an equidistant two-dimensional lattice with lattice constant $10\,\mu m$. This corresponds to the average cell diameter of B-cells in GCs. The radius of the lattice is $220\,\mu m$, corresponding to a typical radius of a GC. Each lattice point can be occupied by exactly one centroblast, centrocyte, or output cell. All cells move on the lattice through diffusion. Other concepts of cell motility in GCs are currently under investigation.[26] The diffusion constants are adapted corresponding to the different diameters of centroblasts and centrocytes.[1,27,28] FDCs are represented by a soma at one lattice point and four (in 2 dimensions) dendritic arms of $30\,\mu m$ length. FDCs are assumed to be immobile which basically has an impact on the interaction frequency with B-cells.[19]

It has been previously shown that the development of dark zones requires a non-local cell-cell interaction.[19] Such an interaction may be provided by a chemotaxis gradient which acts on the motility of centrocytes and stems from FDC and/or T-cells and/or naive B-cells in the mantle zone.[26] Another possibility (which will be used in this work) is a diffusing signal molecule which is produced by FDCs or T-cells and bound by centroblasts.[19] Note, that this implies a separation of signals acting on proliferation and differentiation of centroblasts, as has been proposed in corresponding experiments.[29] The signal molecules are clustered in quanta that diffuse on the lattice according to a classical diffusion equation. The diffusion is not influenced by the presence of cells at the same lattice point. One quantum corresponds to the signal concentration that is necessary to initiate the centroblast differentiation process into centrocytes. Using this non-local concept an intermediate dark zone is produced.[19] The duration of the dark zone is basically depending on the amount of secerned signal molecules and its diffusion constant. The ratio of centroblast differentiation and proliferation rates changes the duration of the dark zone as well. However, this ratio also has influence on the total life time of the GC as a whole and, therefore, is determined independently.

The affinity of the encoded antibodies to the antigen is formulated with the well known shape space concept.[30] Each type of antibody is represented on a four-dimensional lattice which is ordered in such a way, that neighboring points have similar affinity to the antigen. A hypermutation is represented by a jump to a neighbor point. The affinity between the antibodies on a centrocyte and the

antigen on an FDC is modeled by a gaussian affinity weight function centered at the optimal antibody type.[20]

The dynamical properties of the different cells types on the lattice are summarized in the following. On the quantitative level the parameter values have been determined using experimental constraints. In many cases the parameters were directly accessible from experiment. Others had to be determined indirectly using experimental observations of the general GC properties (for more details we refer to[19,20]).

- **Centroblasts** proliferate with a (constant) rate of $1/6\ hr$.[2,31,32] At each division a somatic hypermutation occurs with the probability $1/2$.[33,34] They differentiate in dependence on a differentiation signal that is secerned by the FDCs, and diffuses over the lattice. The differentiation process is activated when a centroblast meets a threshold quantum of differentiation signal at the same lattice point. Activated centroblasts differentiate with a rate of $1/6\ hr$ into centrocytes.[2,35,36] A finite life time of centroblasts is not imposed. However, the effective life time is shorter than 1 day due to centroblast differentiation.

- **Centrocytes** die with a rate of $1/7\ hr$.[27] They bind to the FDCs according to the affinity to the antigen represented by an affinity weight function. Bound centrocytes remain bound for $2\ hr$.[37,38] They are thought to be rescued from apoptosis during this time. Positively selected centrocytes further differentiate with a rate of $1/7\ hr$ into either re-proliferating centroblasts (with probability 80%),[20] or into output cells (with probability 20%). The differentiation into output cells is delayed by $48\ hr$ with respect to the starting time of hypermutation,[5,20,39] i.e. it starts at day 6 of the GC reaction.

- **Output cells** leave the GC by diffusion and do not further interact with other cells in the GC.

- **Dead cells** are eliminated from the GC.

The simulations are started with 3 randomly distributed seeder B-cells and 20 FDCs. The fact that centroblasts proliferate at least in parts outside the FDC network during their proliferation phase turned out to be a necessary requirement for the development of dark zones.[19] This is ensured by a random distribution of the FDCs on 70% of the (maximum) GC volume. The seeder cells are of low but non-vanishing affinity to the antigen. They can reach the optimal antibody-type with 5 to 10 mutations.[4,40] The simulations are insensitive to a change of the time-step-width which is $0.004\ hr$ for the presented results. In a stochastic model the outcome of the simulation depends on the used generator of random numbers and

on its initialization. Therefore, the results are given with a standard deviation corresponding to this uncertainty.

3 Results

At first some basic properties of the GC reaction are reported as generated by the model simulation. Assuming the already introduced centroblast differentiation signal (secerned by FDCs and bound by centroblasts) a dark zone develops. It appears at day 4 of the reaction, and remains stable for a duration that depends on the production rate of the signal molecules. In order to simulate different durations of dark zones, the signal production rate is varied. For each production rate the differentiation rate of centroblast is adjusted correspondingly (within physiological constraints) in order to ensure a comparable final state of the GC after 21 days of the reaction. The development of the dark zone as well as its depletion are not principally affected by the variation of other parameters within their physiological constraints. For example a smaller proliferation rate basically stretches the whole GC reaction without changing the general behavior.

The time course of the total GC volume is in accordance with experimental observations[2,41] provided that the dark zone vanishes between day 6.5 and 10 of the GC reaction (i.e. that the signal production rate is chosen correspondingly): After an exponential increase of the total cell population, a maximum is reached after 4 days of the reaction. The total cell population then is diminished steadily until the end of the reaction after 21 days. At this time only about 50 proliferating B-cells remain in the GC.[2,18] Taking these results together, the general GC morphology is well described by the model results.

The average affinity of B-cells is enhanced during the whole GC reaction. This is best illustrated by looking at a typical example with a dark zone present until day 8.3. In Fig. 1 the time course of the fraction of high affinity centroblasts and centrocytes is shown.[19] *High affinity* denotes those B-cells which bind the antigen with a probability of more than 30%. As can be seen, such a cell does not exist at the beginning of the GC reaction. They develop after the start of somatic hypermutations after 3 days. Still one observes a short delay because some mutations have to occur before the first cells appear that have an above threshold affinity to the antigen. Then the relative number of high affinity cells grows steadily. In accordance with experiment, good cells already dominate around day 10 of the GC reaction.[5] One observes an intermediate steep increase, that approximately starts when the dark zone is depleted. This correlation does not seem to be obvious in view of this single example. However, it has been observed in all simulations, especially in those with different dark zone durations. In the late phase of the GC reaction the curve reaches a plateau on a high level, and

practically all B-cells are high affinity cells.

In the same figure the fraction of high affinity output cells is shown. This curve does not show the value at each moment of the GC reaction but the sum of all output cells that has been produced until time t. This value is a measure for the total quality of the produced output cells. When the first high affinity output cell is produced at day 5 of the reaction, the total output quality is increased according to its affinity. Subsequently, the quality of the output cells is steadily increased during the GC reaction. The part of high affinity output cells reaches 77% in the present example. The average over all simulation with dark zones that vanish between day 8.5 and 10.5 of the reaction is $75.2\% \pm 4.2\%$ (the error is one standard deviation). In view of the fact that at the beginning of the GC reaction no high affinity B-cell existed at all, this affinity enhancement is remarkable.

The main task of the present article is to analyze a possible correlation of the duration of the dark zone and the achieved affinity maturation, i.e. the total output quality at the end of the reaction. This analysis has to be based on comparable GC simulation. By changing the production rate of the centroblast differentiation signal molecule not only the duration of the dark zone is varied. Also the total duration of the reaction is changed. As stated at the beginning of this section, the centroblast differentiation rate is adjusted correspondingly so that the final numbers of B-cells N after 21 days of the reaction are of the same range. This is especially important as the affinity maturation process depends on the value of N.[19] The average number of B-cells for all 226 simulations is $\overline{N} = 49 \pm 23$. The error denotes one standard deviation. Only those simulations are taken into account that generate a final number of B-cells N within one standard deviation of this average value. The remaining simulations are grouped according to the duration of the dark zone (see Tab. 1). Note, that due to the described procedure the simulations within each group have similar average N.

The output quality is plotted against the duration of the dark zone (see Fig. 2). The quality of the produced output cells becomes optimized for dark zones that vanish between day 7 and 11 of the GC reaction. For shorter and longer dark zones, the resulting quality of the output cells is reduced. It can be deduced from Tab. 1 that the same holds true for the total number of output cells.

4 Discussion

We used a previously developed model to elucidate a possible correlation between the duration of the dark zone and the efficiency of the affinity maturation process. In a first step the simulated GCs were compared to GCs observed in experiment. The essential properties of real GC reactions were reproduced correctly. This includes the appearance of the dark zone, the time course of the total volume, as

well as the reached affinity maturation.

It has been previously found[19] that dark zones do not appear in simulations that are based on local cell interactions only. Therefore, it was necessary to introduce an non-local cell interaction into the model in order to understand the intermediate appearance of dark zones as observed in experiment.[17] This has been realized using a centroblast differentiation signal molecule that is secerned by FDCs and consumed by the centroblasts. Such a signal molecule has to be understood as a hypothesis, because it still remains unclear how the centroblast differentiation process is initiated in real GC reaction. However, there are experimental hints that centroblast differentiate due to an interaction with FDCs or T-helper cells.[29,42,43]

Most parameters in the model are strongly constrained by experimental data,[19] so that the possiblity of varying the parameters is restricted. However, the production of the differentiation signal (as not directly observed in experiment) does not underly such a restriction. It turned out that the duration of dark zones strongly depends on the production rate of the differentiation signal. The duration of the dark zone is most sensitive to this rate compared to other model parameters. This situation opens the possibility to vary the signal production rate and in this way to test the affinity maturation process of GCs with dark zones of various duration. A corresponding test is not possible in experiment as in experiments one is restricted to GCs that are realized in nature.

A statistical analysis of simulations with various durations of dark zones (i.e. various production rates of the differentiation signal) leads to the conclusion that the quality of the output cells averaged over all produced output cells during the whole GC reaction is optimized for intermediately appearing dark zones vanishing between day 7 and 11 of the GC reaction. Note that also the quantity of produced output cells is optimized for those dark zone durations. In addition, the time course of the total GC volume is in agreement with time courses observed for the same dark zone durations.[19] These are also typical dark zone durations as they have been observed in experiment.[17]

This result suggests a relation of two, at first sight, very different categories: The GC morphology and affinity maturation of B-cells. The morphology of the GC is basically determined by a non-local interaction with other cell-types. However, the function of the specific GC morphology is not restricted to a spatial arrangement of interacting cells. One may suspect that specific cell arrangements are advantageous for the success of the GC reaction. The duration of the dark zone does not only seem to *describe* the time course of the cell distribution in GCs but also to be a critical parameter for the quality and amount of output of the GC reaction. This leads to the question of how the spatial distribution of centroblasts and centrocytes influences the affinity maturation process.

On one hand the existence of the dark zone is necessary to produce a large

pool of different B-cell types, i.e. a high diversity of encoded antibody-types. This diversification is optimally realized with a fast proliferation of centroblasts without major interaction to antigens. The mutations are likely to occur randomly[44] and it is supposed that starting from low (but non-vanishing) affinity seeder cells better B-cells are found during this process. The diversification process continues in the dark zone when the selection process has already started. Note that recycled B-cells that return into the dark zone don't have been observed in the simulations.[19] Therefore, the B-cells that proliferate in the dark zone are not recycled B-cells but directly stem from the original seeder cells.

After about 8 days some high affinity cells have been found and identified in the selection process that takes place in the light zone. Those roughly optimized B-cells are mostly recycled cells[45] and restart to proliferate. A further enhancement of affinity to the antigen is based on these preselected B-cells. One may think of an ongoing process of diversification in the dark zone. However, the cells produced in this random process cannot overcome a certain B-cell quality. Therefore, a re-proliferation of already selected B-cells is more promising for the further development of the GC reaction. This process resembles a directioned selection process that replaces the random process in the dark zone. Ongoing proliferation and mutation in the dark zone do not only become senseless (as the quality of the cells in the dark zone are below the average B-cell quality in the light zone). They would also hinder the optimization process in the light zone because B-cells of relatively low affinity would take part in the selection process. Consequently, the selection of high affinity cells would be inhibited by a senseless repetition of a first selection process with B-cells stemming from the dark zone. An early depletion of the dark zone eliminates the low affinty B-cells from the GC and in this way allows a fine-tuning of preselected B-cells.

This interpretation of the necessity of the early depletion of dark zones in GC reactions is directly coupled to the cell distribution in GCs, i.e. to its morphology. A diversification is possible only if the centroblasts proliferate and mutate outside the FDC network. Later in the GC reaction a fine-tuning of already preselected B-cells has necessarily to take place in the direct neighborhood of the antigen presenting FDCs. The existence of a dark zone inhibits affinity maturation in this phase and a homogeneous distribution of centroblasts and centrocytes in the FDC network is more advantageous for the development of the GC reaction.

One should be aware that this interpretation only provides a possible explanation of the correlation between morphology and affinity maturation that has been found in the framework of a spatial model for GCs. The basic concepts used here are in accordance with a widely accepted picture of GCs. However, a possible function of the specific morphology of GCs for the success of GC reactions is an important part of the understanding of processes inside GCs.

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Figures and Tables

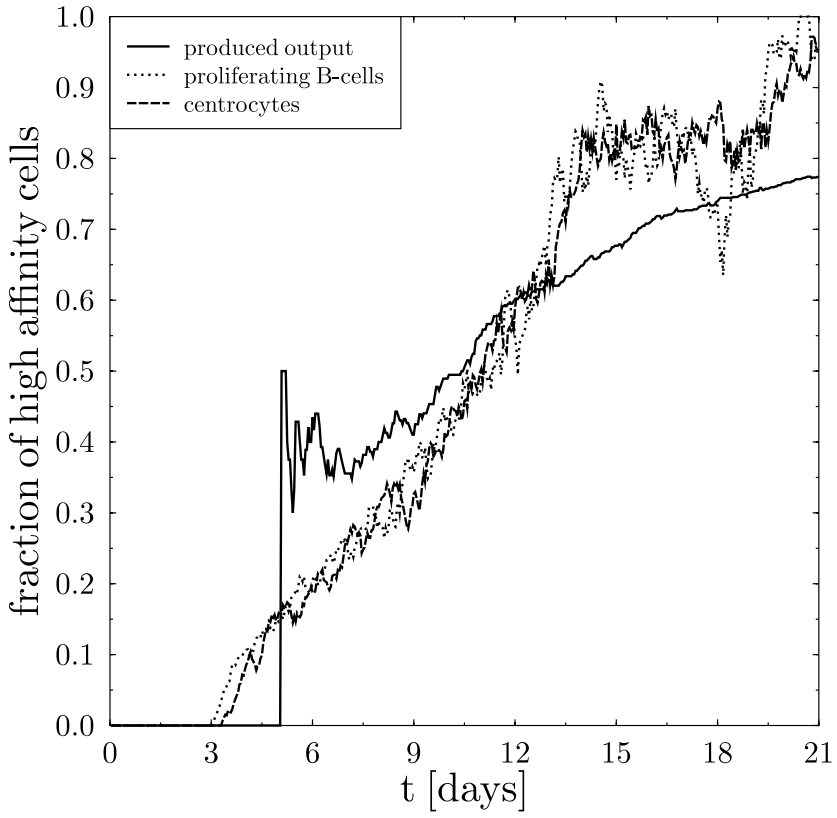

Figure 1: Affinity enhancement:
The time course of the fraction of high affinity cells (cells which bind the antigen with a probability of at least 30%) in the GC reaction is shown for centroblasts, centrocytes, and for the sum of all output cells produced until time t of the GC reaction.

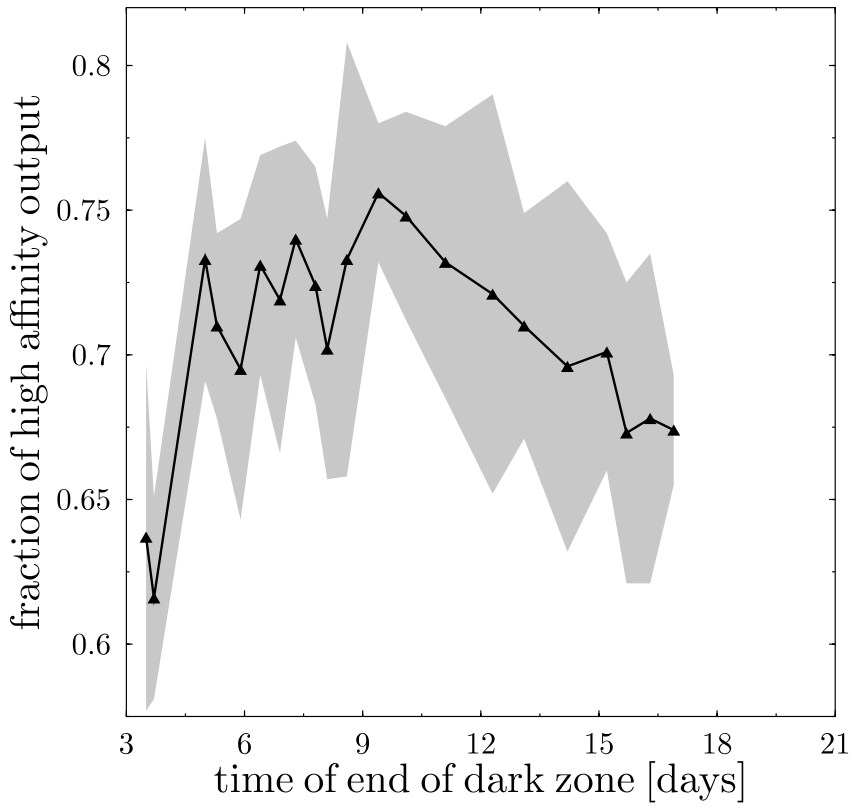

Figure 2: The importance of the dark zone duration:
The dependence of the fraction of high affinity output cells on the duration of the dark zone. The grey area denotes one standard deviation of the average values (full line).

n	T	N	O	f
7	3.5 ± 0.0	56 ± 12	185 ± 18	63.7 ± 6.0
9	3.7 ± 0.1	43 ± 13	175 ± 25	61.6 ± 3.5
9	5.0 ± 0.1	43 ± 11	218 ± 28	73.3 ± 4.2
7	5.3 ± 0.1	47 ± 13	205 ± 29	71.0 ± 3.2
8	5.9 ± 0.1	47 ± 12	216 ± 35	69.5 ± 5.2
9	6.4 ± 0.2	47 ± 13	225 ± 22	73.1 ± 3.8
9	6.9 ± 0.1	47 ± 10	219 ± 31	71.9 ± 5.3
8	7.3 ± 0.1	44 ± 12	234 ± 25	74.0 ± 3.4
8	7.8 ± 0.1	51 ± 14	242 ± 37	72.4 ± 4.1
9	8.1 ± 0.1	44 ± 8	221 ± 32	70.2 ± 4.5
6	8.6 ± 0.1	47 ± 15	236 ± 32	73.3 ± 7.5
7	9.4 ± 0.2	51 ± 14	256 ± 29	75.6 ± 2.4
9	10.1 ± 0.2	51 ± 16	246 ± 39	74.8 ± 3.6
9	11.1 ± 0.2	50 ± 13	237 ± 21	73.2 ± 4.7
9	12.3 ± 0.3	49 ± 15	266 ± 34	72.1 ± 6.9
8	13.1 ± 0.2	52 ± 17	243 ± 27	71.0 ± 3.9
7	14.2 ± 0.3	48 ± 14	229 ± 32	69.6 ± 6.4
9	15.2 ± 0.2	48 ± 5	224 ± 27	70.1 ± 4.1
6	15.7 ± 0.2	42 ± 10	216 ± 26	67.3 ± 5.2
8	16.3 ± 0.2	56 ± 12	229 ± 25	67.8 ± 5.7
5	16.9 ± 0.1	59 ± 13	221 ± 11	67.4 ± 1.9

Table 1: The sequence of data points in Fig. 2. All simulations that end up with 49 ± 23 B-cells at day 21 of the GC reaction (this is the average value of all performed 226 simulations) are taken into account. The simulations are grouped according to the dark zone durations T (in days). Each value of T corresponds to one data point in Fig. 2. The number of simulations n and the average number of remaining B-cells N within these simulations are given for each data point. The fraction of high affinity output cells f (in $\%$) at day 21 of the GC reaction and the total number of produced output cells O are given. The errors correspond to one standard deviation.